Evaluating Potency of Live Attenuated MTB (AMTB) Trivalent Vaccines for TB, Malaria, and HIV

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Take Home Messages

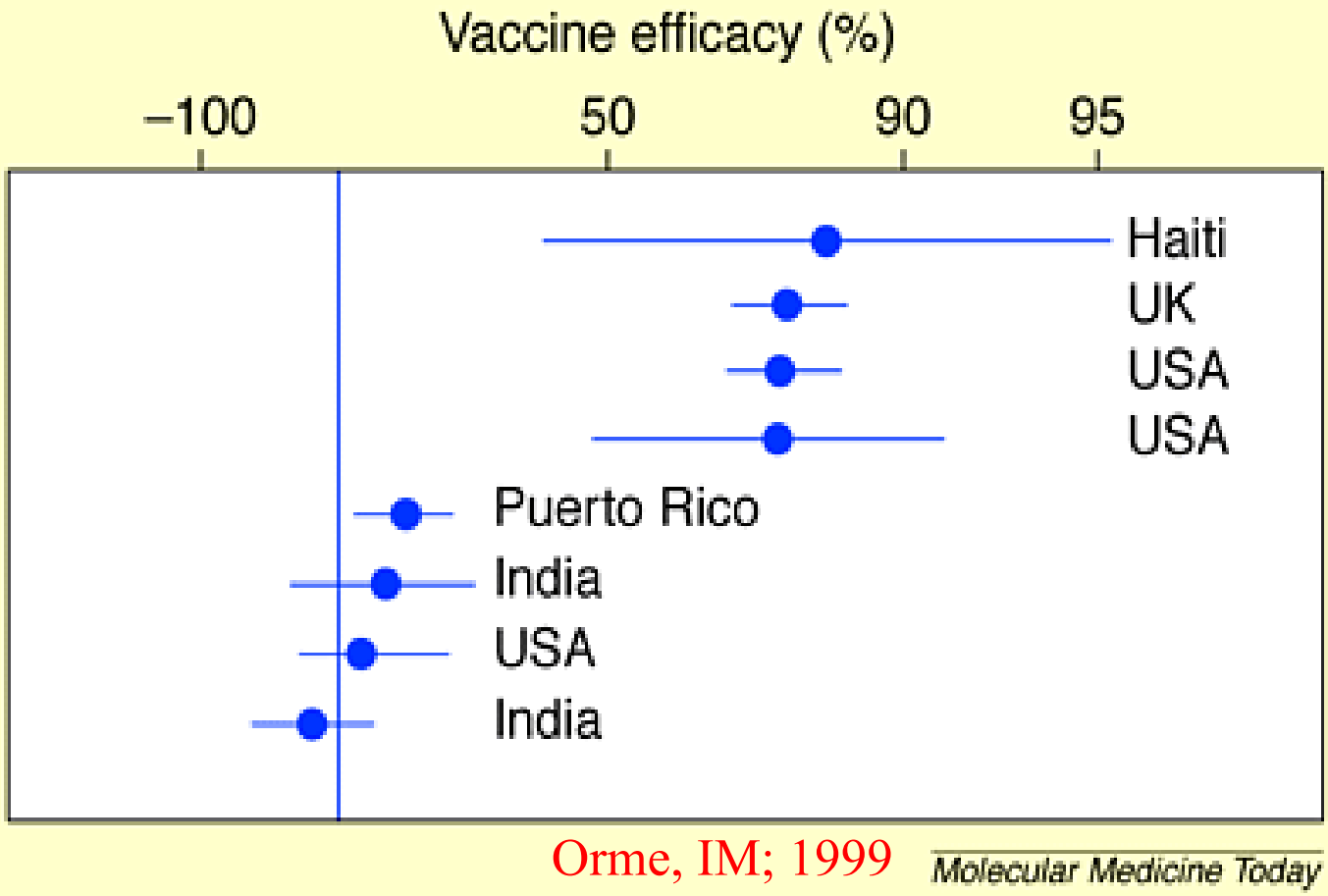
- Attenuated MTB strains that are safe and Immunogenic can be made easily.
- Protection against TB might follow typical immunological paradigms.
- CTL-enhancing mutants can be made.
- Surrogate Pathogens can be used to help evaluate Vaccine candidates.

Advantages of Recombinant BCG

(Jacobs and Bloom; *Nature* 1987)

- Only live vaccine currently given at birth
- Safe -Over 3 billion doses have been administered
- Can provide long lasting immunity
- Mycobacterial Cell Walls are the adjuvant component of Freund's complete adjuvant.
- Can be an efficient part of prime and boost
- Genetically tractable

BCG Vaccine Efficacy



Derived from Rodrigues & Smith, 1990.

Hypothesis

An *Mycobacterium tuberculosis* derived vaccine can protect better than BCG.

Live Attenuated *M. tuberculosis* Vaccines

- Advantages
  - Low Cost
  - Usable at Birth (Prime of Prime-Boost)
  - Stability
  - Broad Antigen Set (Proteins, Carbohydrates, Glycolipids
- Disadvantages
  - Safety
  - Poor Immunogenicity (immune Evasion)

AMTB – Attenuated *M. tuberculosis*

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Non-replicating Mutants

1. mc^{2}6020 *M. tuberculosis* H37Rv Δ*lysA* Δ*panCD*::*hyg*
2. mc^{2}7002 *M. tuberculosis* H37Rv Δ*lysA* Δ*panCD*
3. *M. tuberculosis* H37Rv Δ*leuD* Δ*panCD*

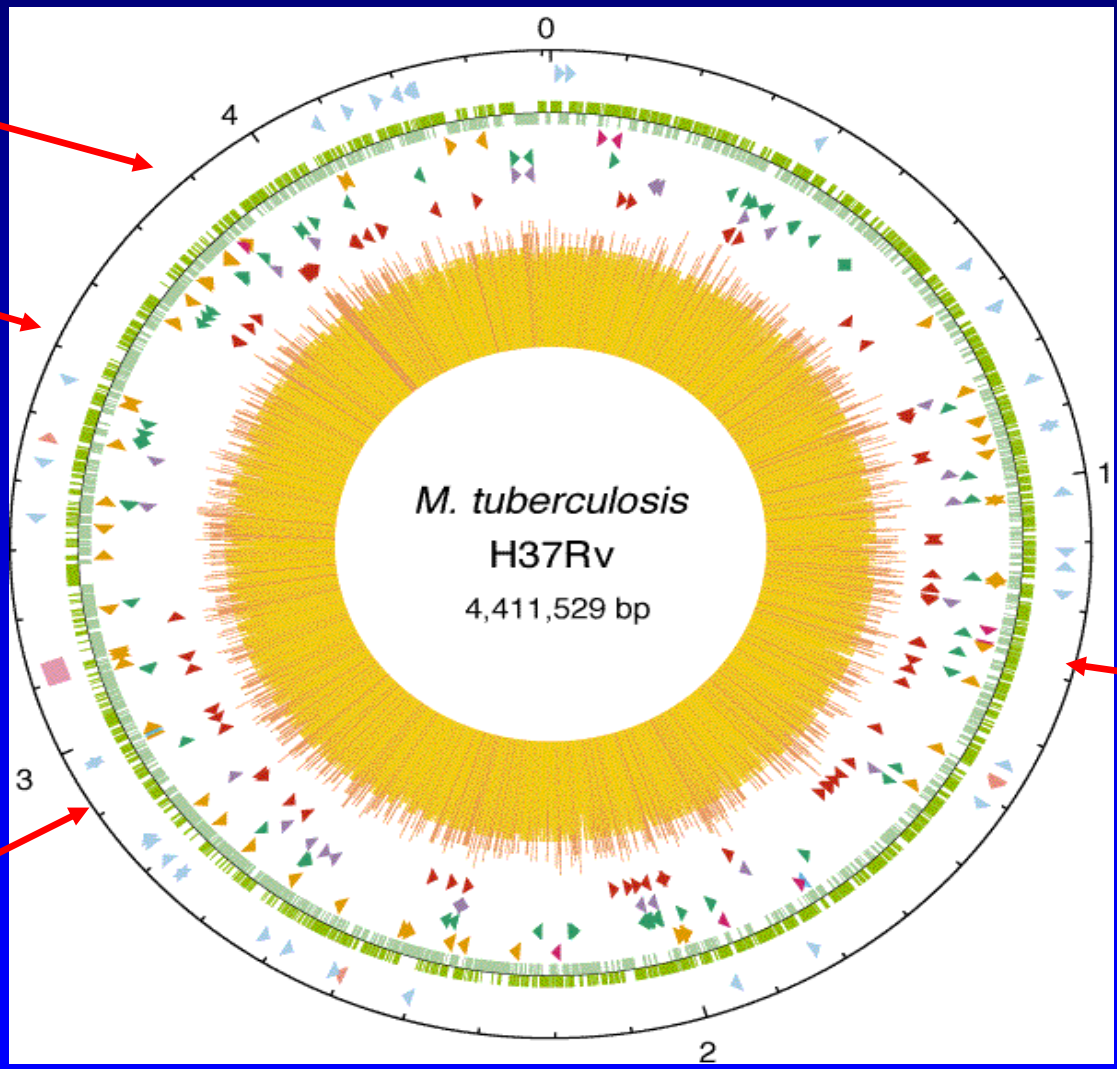
Replicating Mutants

1. mc^{2}6030 *M. tuberculosis* H37Rv Δ*RD1* Δ*panCD*::*hyg*
2. mc^{2}7000 *M. tuberculosis* H37Rv Δ*RD1* Δ*panCD*

Δ*RD1*primary attenuating mutation of BCG

Δ*panCD* – deletes two pantothenate biosynthetic genes

Genomic location of the various deletions in the genome of *M. tuberculosis* H37Rv



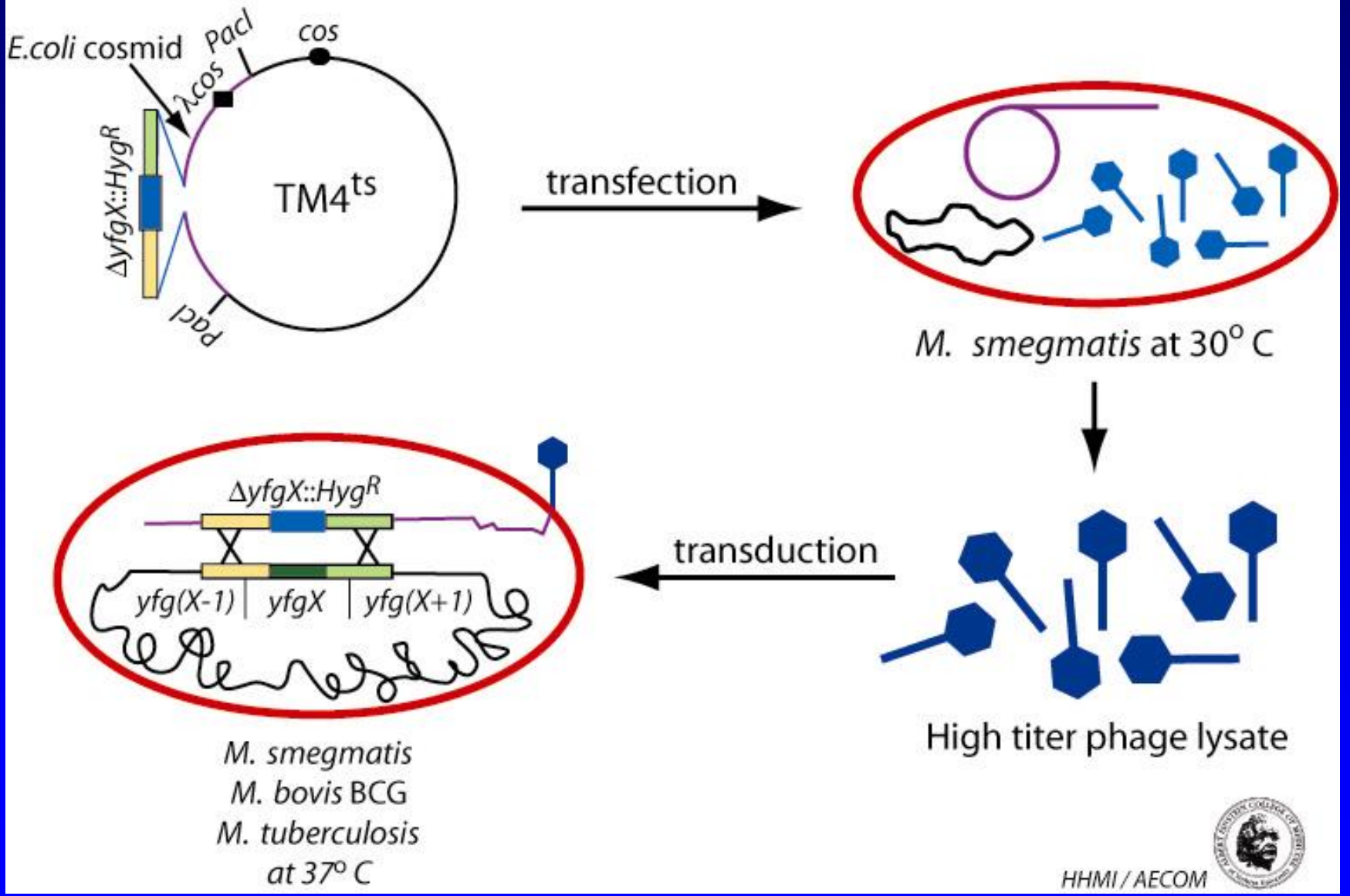
RD1
(Rv3871-79)

panCD
(Rv3602 -03c)

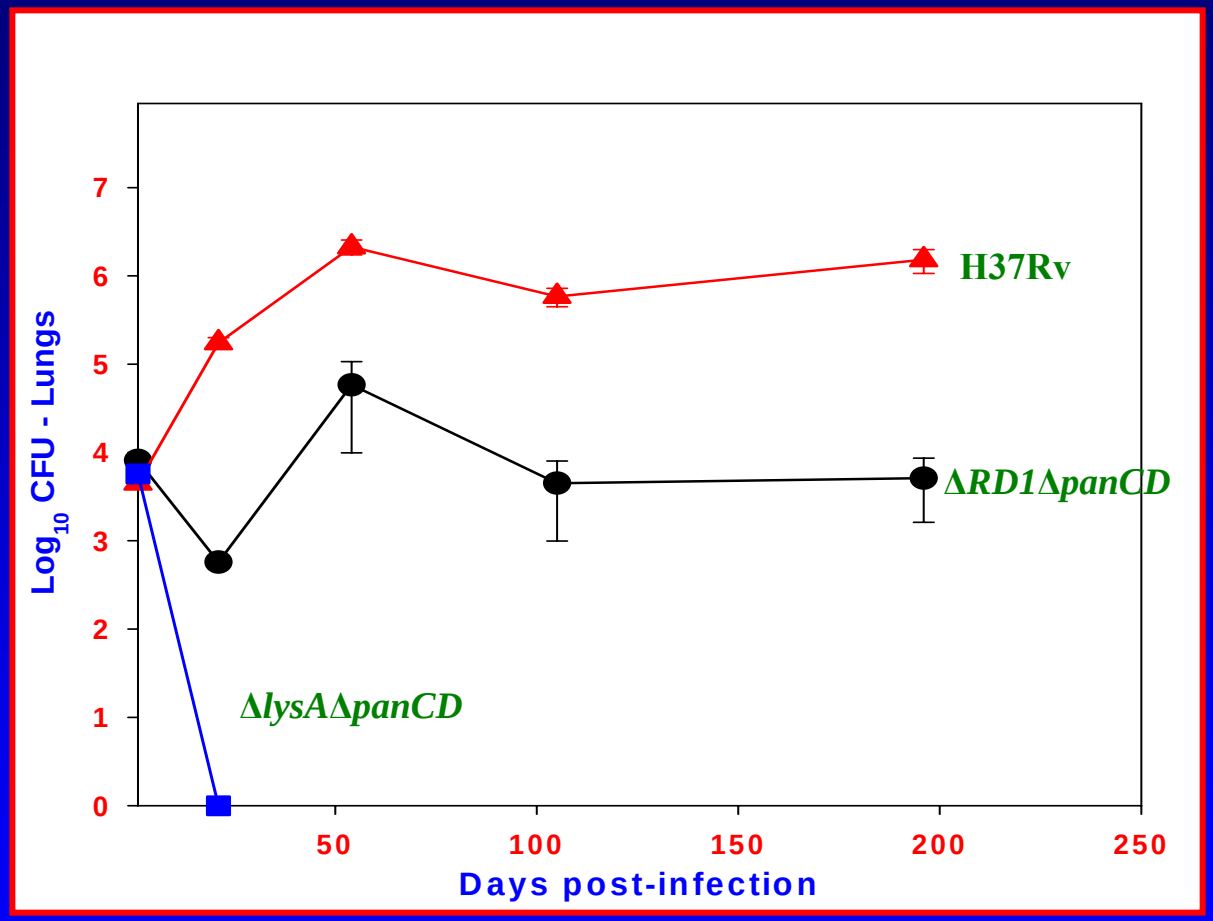
leuD
(Rv2987c

lysA
(Rv1293

SPECIALIZED TRANSDUCTION



Growth kinetics of double deletion mutants in the lungs of C57BL/6 mice

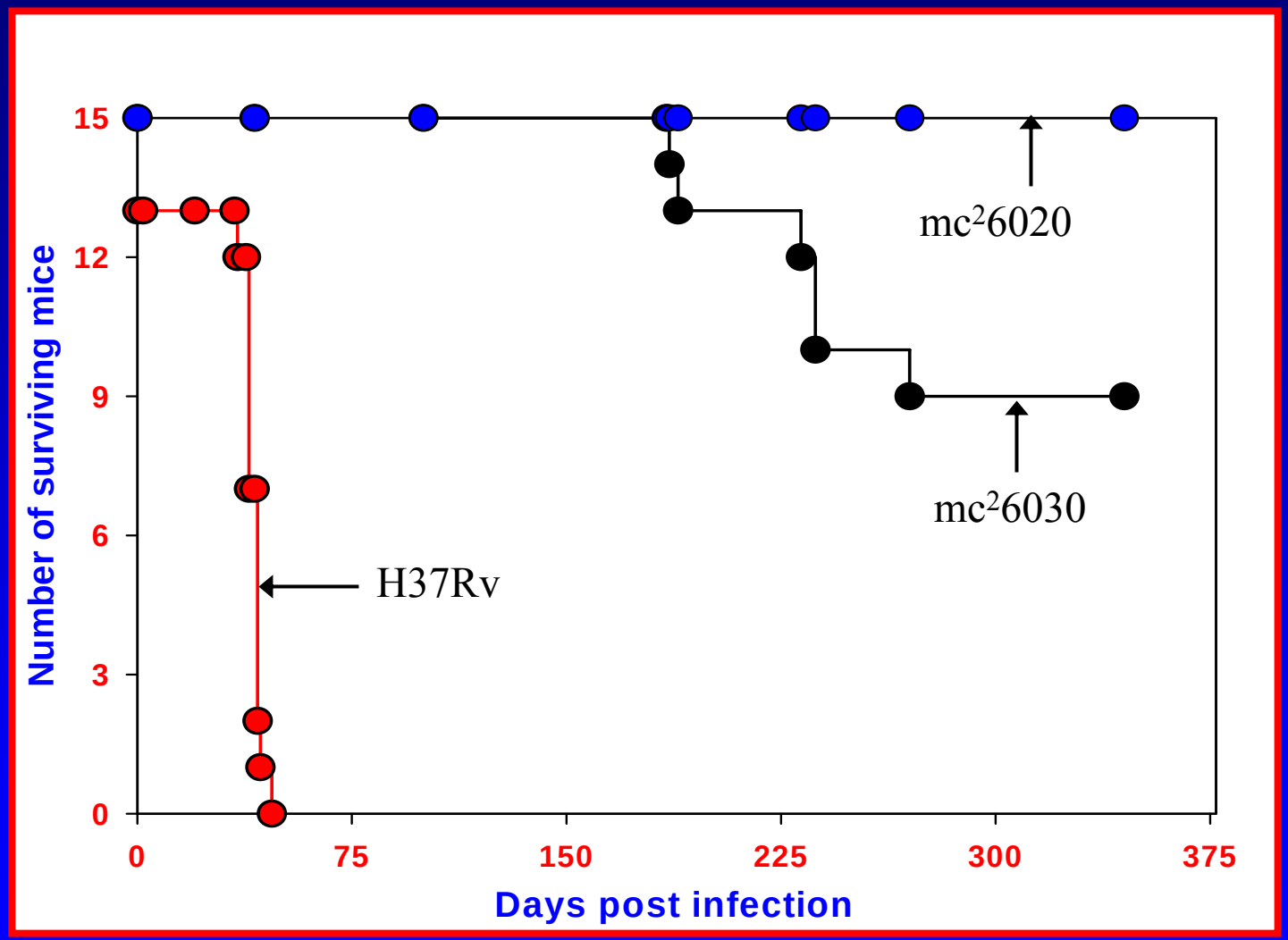


10^6 CFU – i.v. route

Is the vaccine safer than BCG?

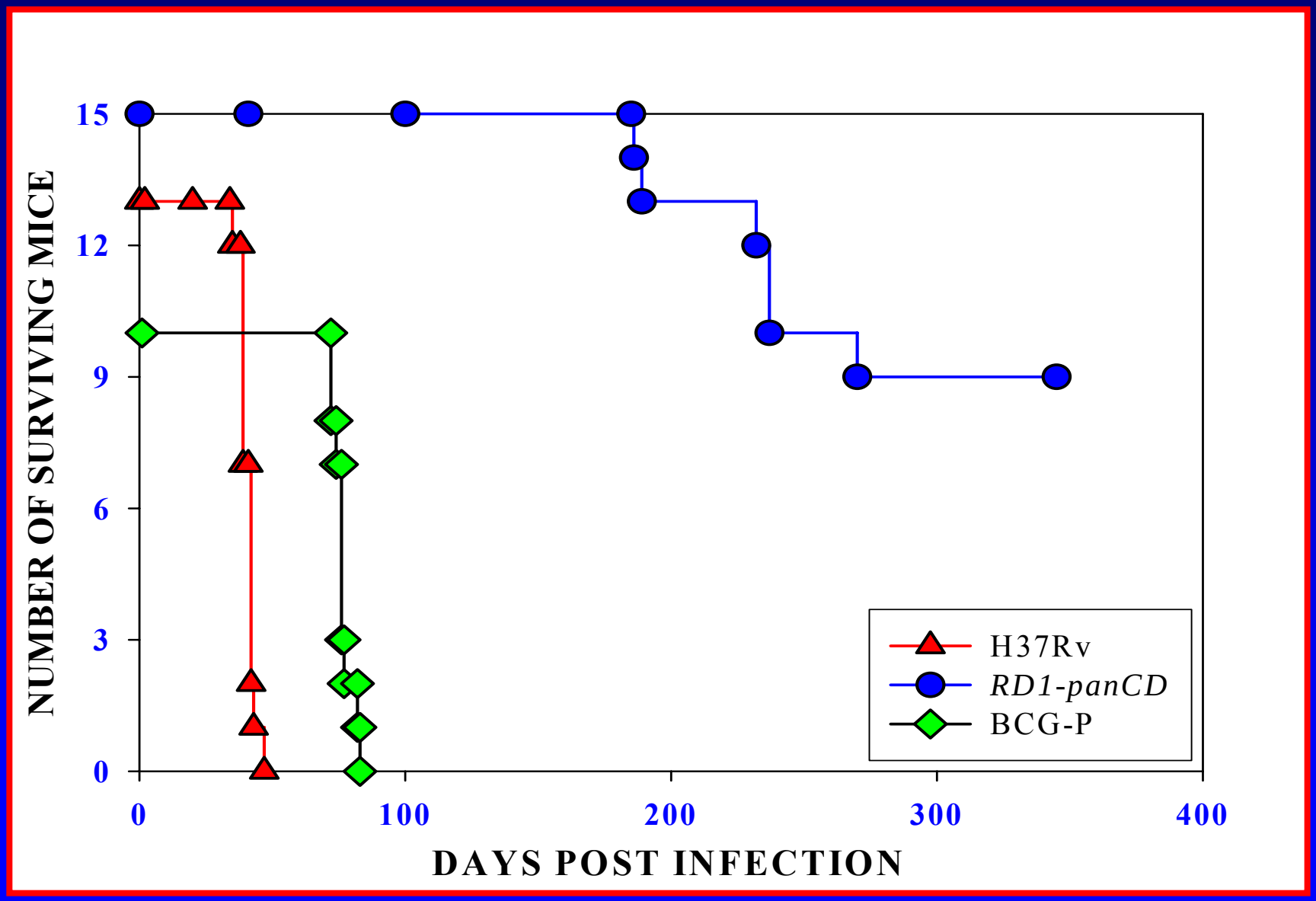
- Intravenous injection of Immunocompromised Mice
- Measure Time to Death
- Very stringent Model of Safety

mc²6020 and mc²6030 are highly attenuated in SCID mice

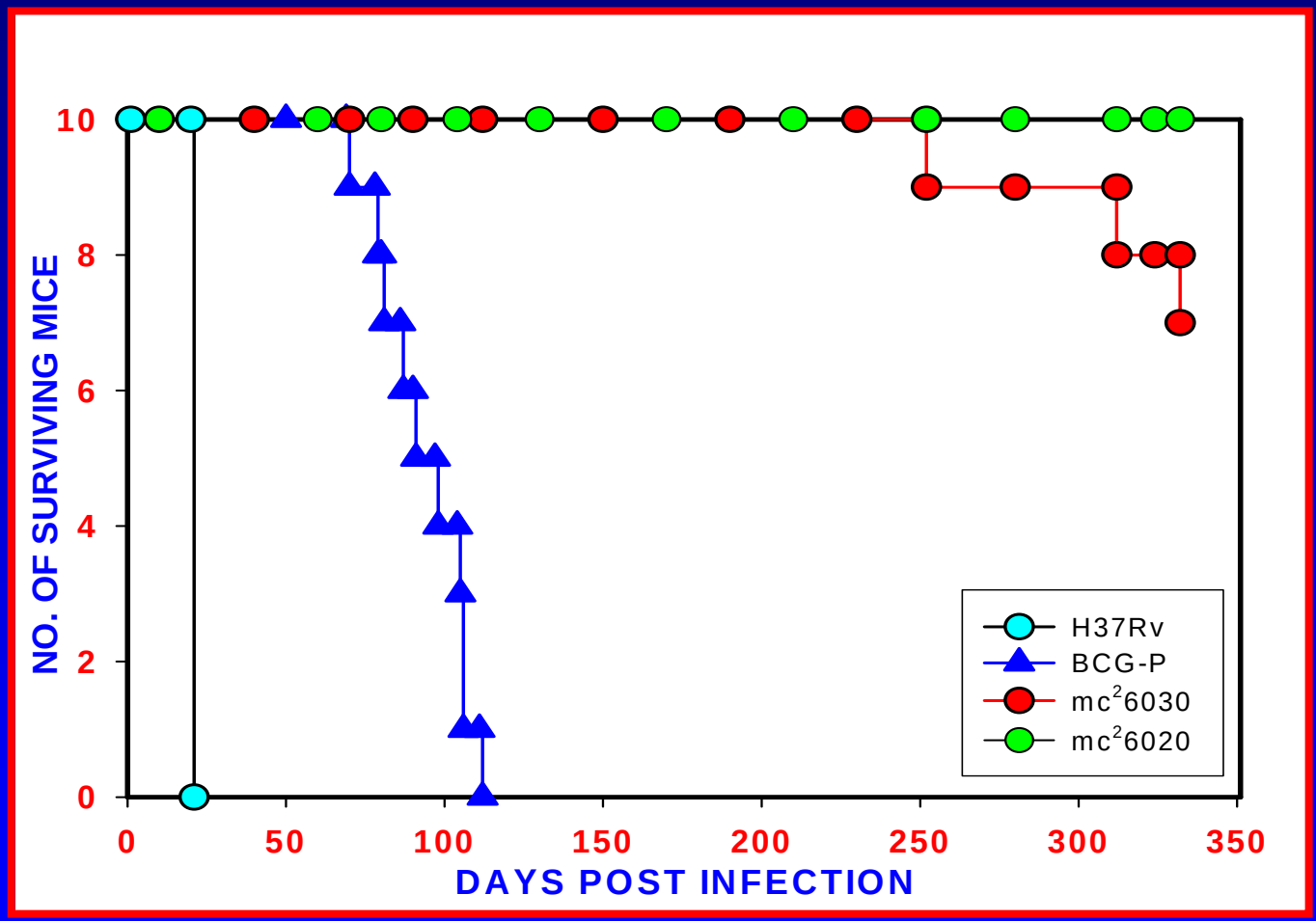


H37Rv: 10^2 CFU; mc²6020 or mc²6030: 10^5 CFU – i.v. infection

M. tuberculosis ΔRD1-Δ*panCD* is highly attenuated in SCID mice



$mc^2$6020 and $mc^2$6030 are highly attenuated in γ-interferon deficient (GKO) mice



10^5 CFU – i.v. infection

Conclusion

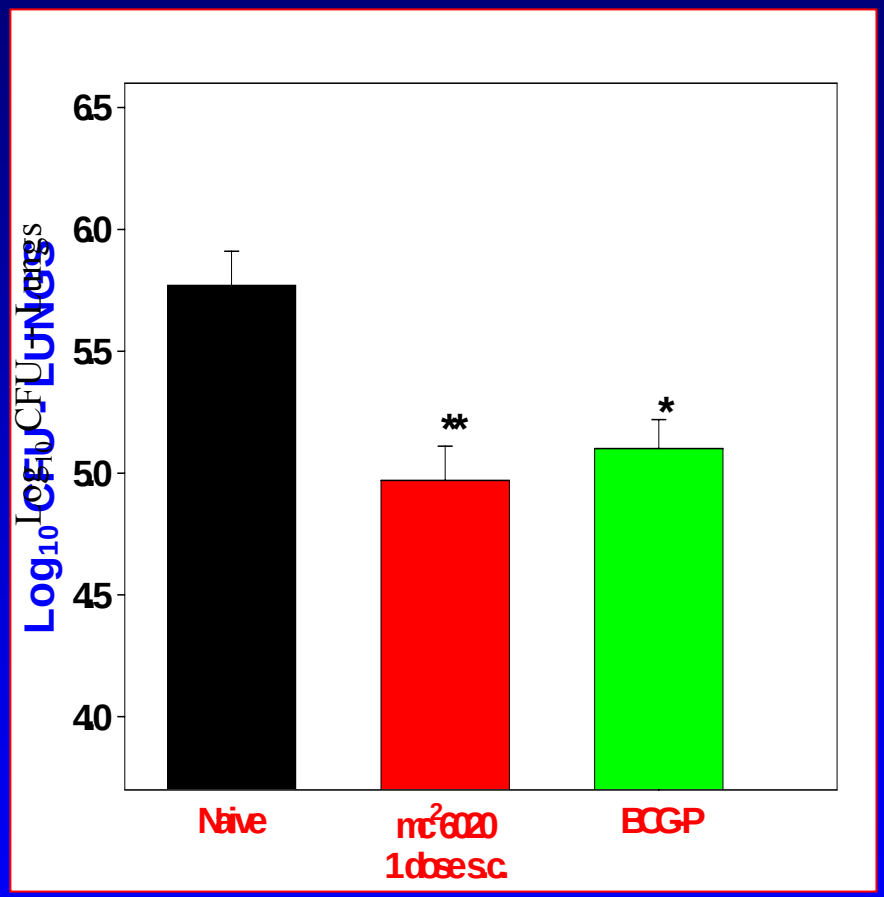
- AMTB strains $mc^2$6020 and $mc^2$6030 are safer than BCG when evaluated in immunocompromised mice.

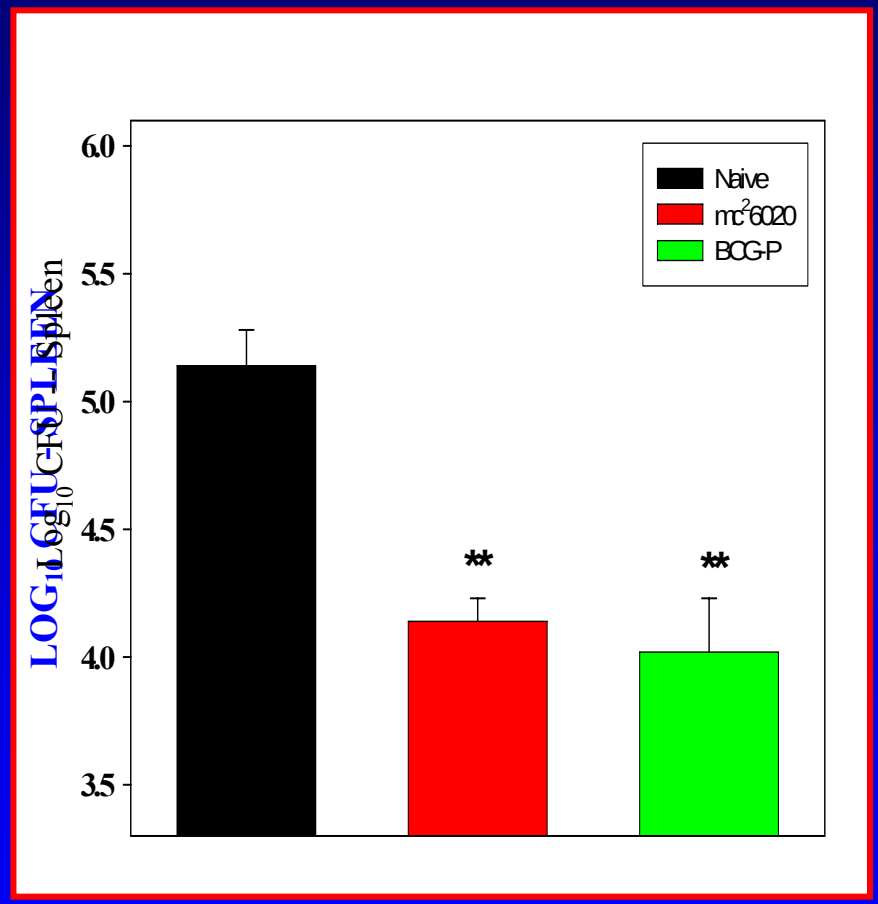
MTB Aerosol Challenge Protection Model

- Single dose subcutaneous immunizations. BCG is given ID, no IV.
- Wait 100 or 240 days.
- Challenge with 100 to 200 virulent *M. tuberculosis*.
- Measure CFUs in the lung and spleen at 28 days post challenge.
- Measure time of Survival for Mice

Long-term protection induced by mc²6020

28 days CFU post-aerosol challenge – C57Bl/6 mice

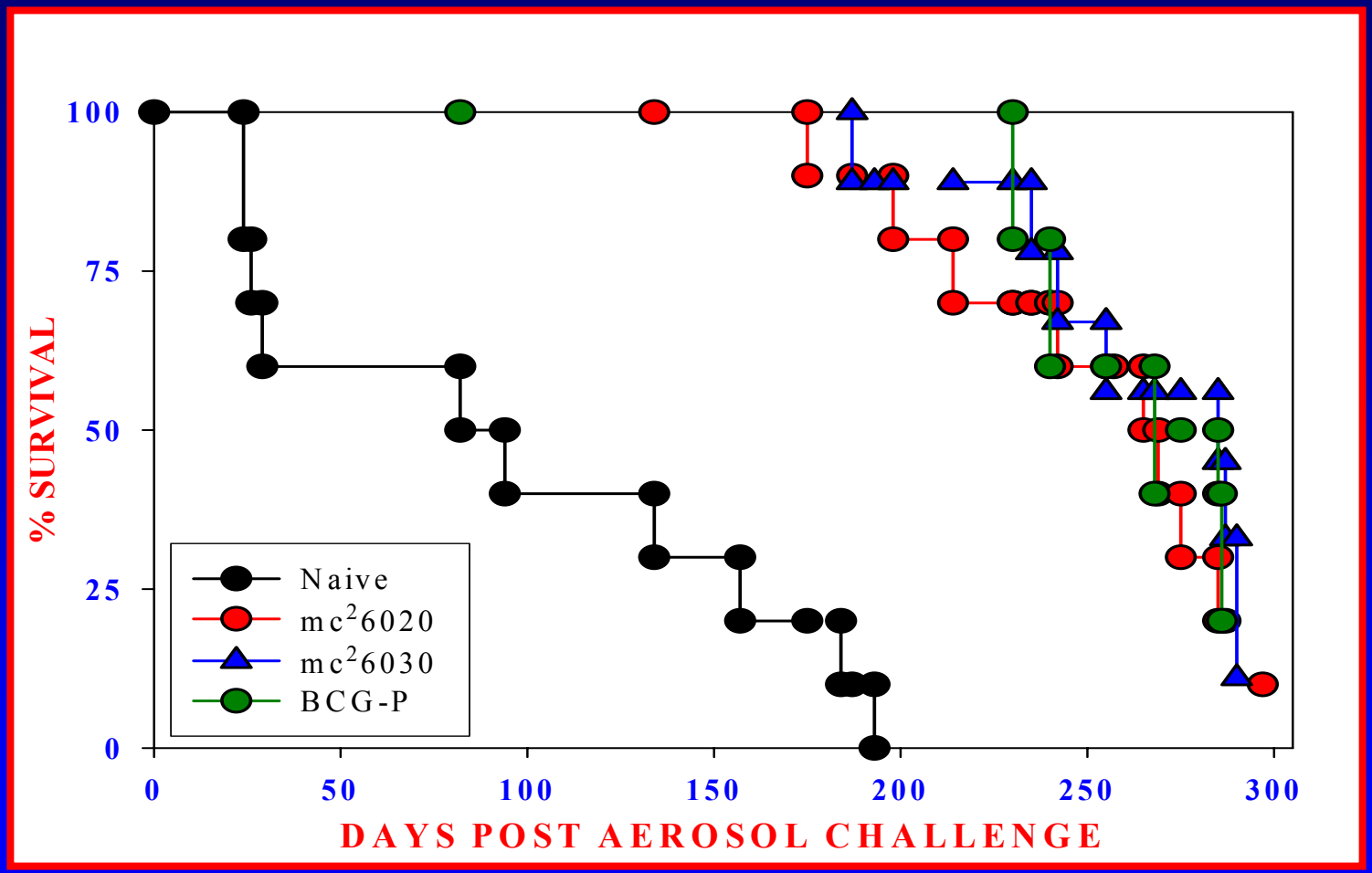




Subcutaneous vaccination - Aerosol challenge after 8 months

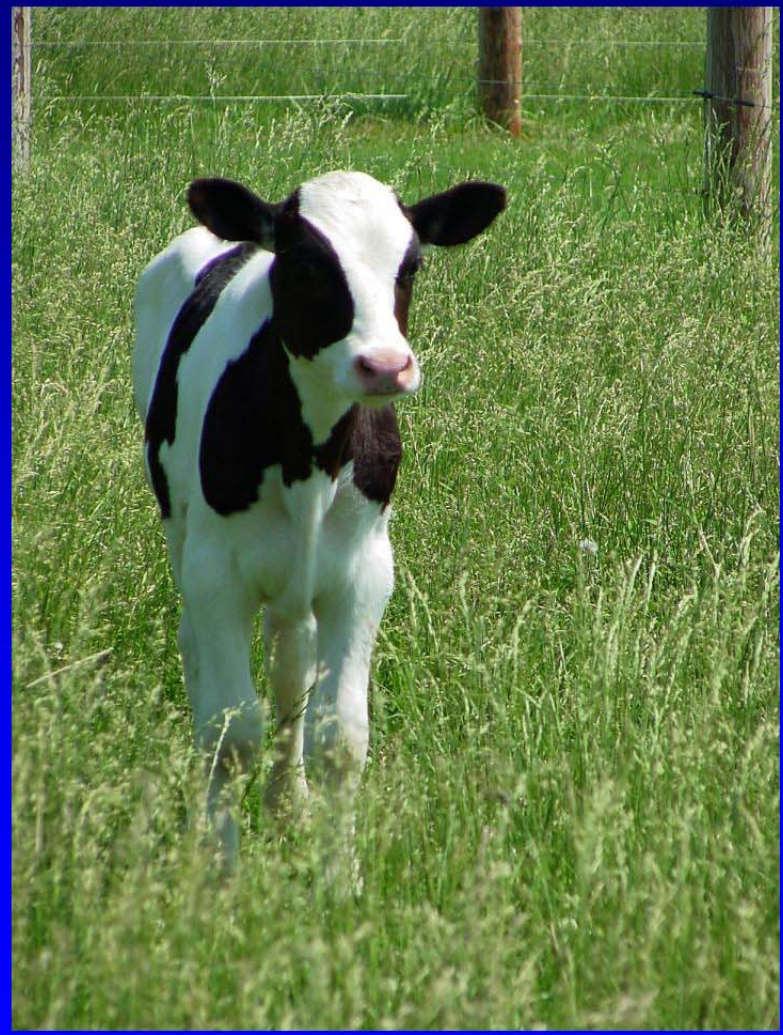
* $P < 0.05$, ** $P < 0.01$ relative to control

Vaccination with mc²6020 or mc²6030 confers long-term survival in C57BL/6 mice



Single dose subcutaneous vaccination - Aerosol challenge after 3 months ** ***P* < 0.001 relative to control**

Pre-Clinical Studies



Safety Studies in Animals

- *Guinea Pigs:* $mc^2$6020 and $mc^2$6030 are safe in the Freedom from Virulence assay (former FDA requirement for BCG vaccines) [Spring Valley Laboratories]
- *Non-Human Primates:* $mc^2$6020 and $mc^2$6030 have been shown to be safe following intradermal immunization with 50-times the human dose in cynomolgus monkeys [Tulane National Primate Research Center]
- *Neonatal Cattle:* $mc^2$6030 is being evaluated for safety and protection in Holstein calves [USDA Tuberculosis Research Center]

Mouse Models for HIV-infected Individuals

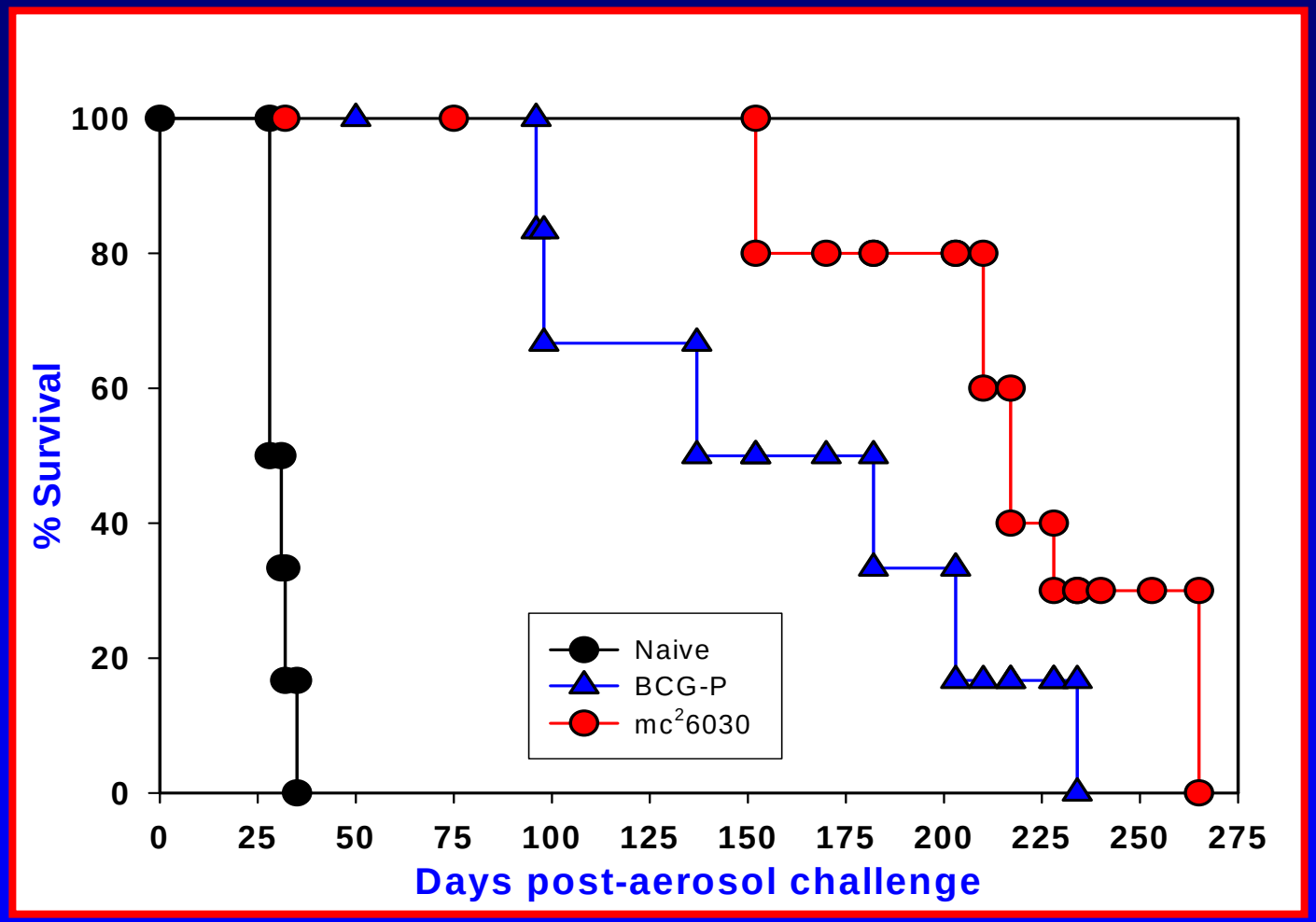
Steve Derrick, Vasan Sambandamurthy, and Sheldon Morris

What is the best model?

HIV-infected mice?

CD4-/- deficient mice?

$mc^2$6030 protects $CD4^{-/-}$ mice from tuberculosis better than BCG



Conclusion

- Protection against TB can be mediated in the absence of CD4, CD8, NK-T cells, and Gamma delta T- cells.

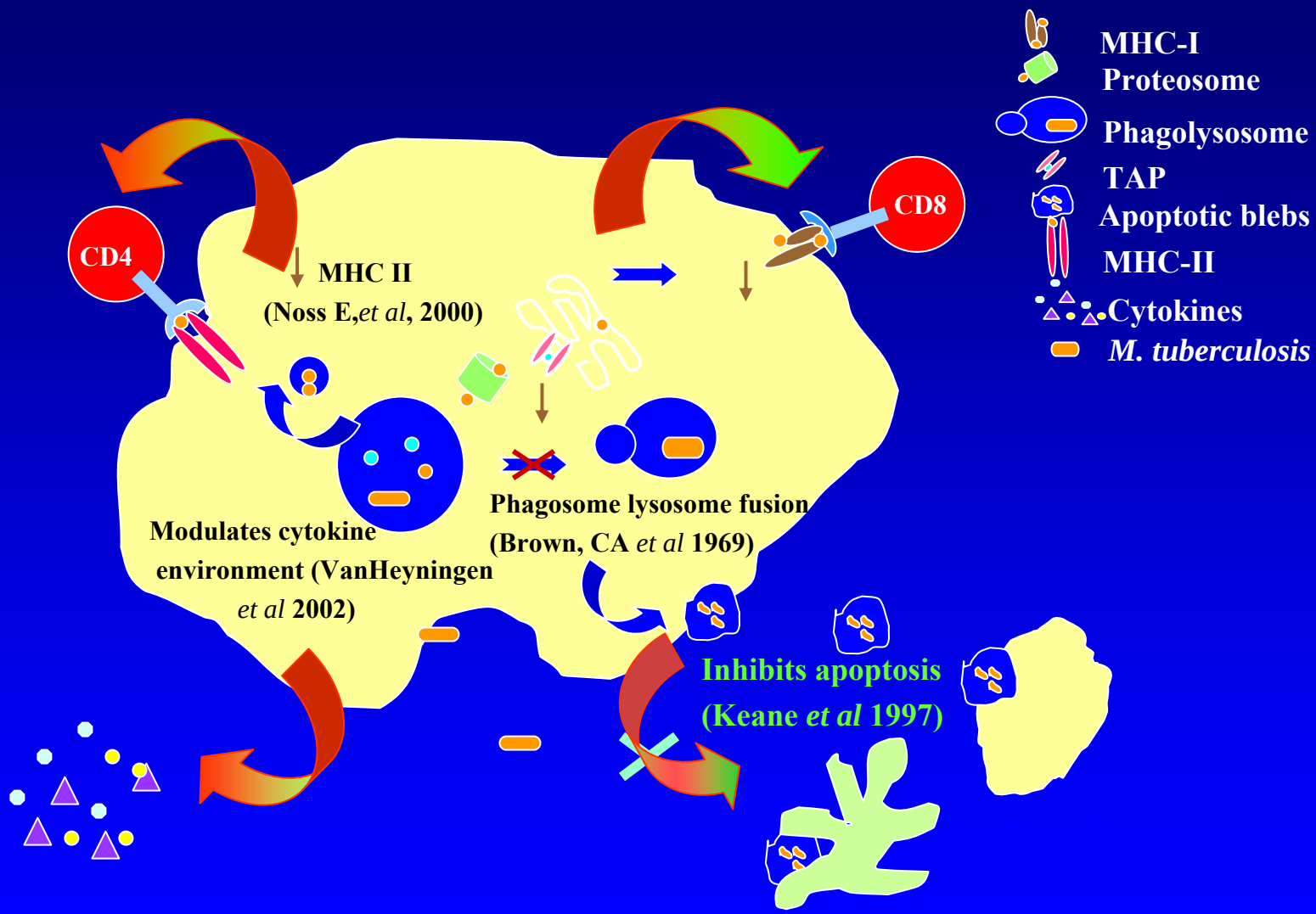
$mc^2$6020 and $mc^2$6030 Summary

- Δ*RD1* Δ*panCD* deletions or Δ*lysA* Δ*panCD* or of the H37Rv sequenced strain
- Undergoes very limited or limited replication in mice
- Safer than BCG in immunocompetent and immunocompromised mice
- Safe in Guinea Pigs, and Cynomologous Monkeys
- $mc^2$6030 is safe in neonatal cows
- Protects Immunocompetent and CD4-deficient mice against *M. tb* challenge
- Elicits Protection, in contrast to DNA immuization, in CD4-deficient mice is mediated by a CD4-negative, CD8-negative, αβ+ T cell
- Stimulated T-cell responses in Cynomologous monkeys
- Expression host for HIV and Malaria Antigens

Hypothesis

Inactivation of Immune Evasion Functions will enhance immunogenicty of recombinant mycobacterial vaccines.

Strategies for Immune Evasion



Hypothesis: Mutants of *M. tuberculosis* defective in apoptosis-inhibition will be more immunogenic

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Steve Porcelli
John Chan

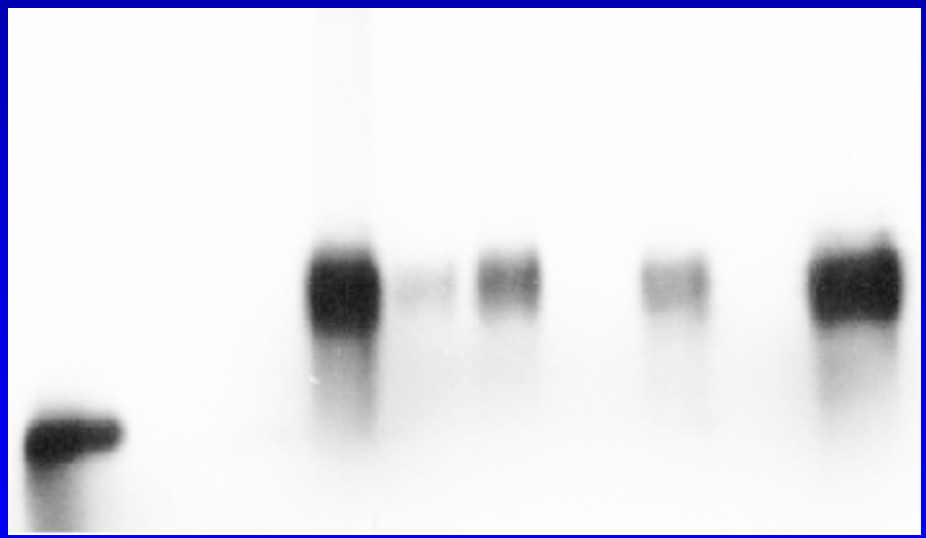
Summary

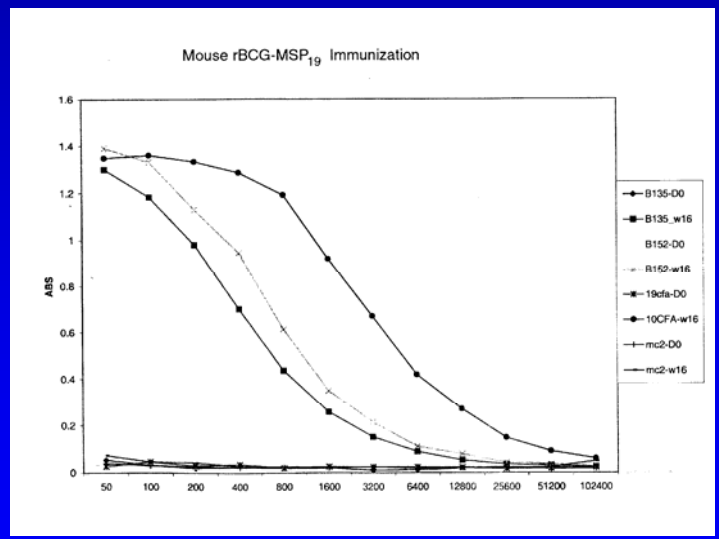
- AMTB can be used to express and prime immune responses to foreign antigens.
- Surrogate Antigens can be highly effective in ascertain specific immunological correlates.
- Four independent mutants have been identified that present CTL antigens better than BCG.
- It is possible to engineer AMTB to enhance priming against Class I-presented CTL antigens.

rAMTB Expressing Malarial antigens

Goal: To prime children against malarial antigens at birth

Msp-1_{19} Expressed in BCG elicits abs Responses





Surrogate Pathogens

- Recombinant Antigens Expressing Human and Non-human Primate precisely defined T-cell and Humoral Antigens.
  - SHIV
  - Recombinant MTB
  - Recombinant *Plasmodium falciparum*

Optimizing Potency of the Trivalent TB, Malaria, and HIV vaccines

- Protective Correlates of Protection for All Three Diseases are poorly understood.
- Precisely defined antigens (MHC Class I, MHC Class I, and CD1) can aid in characterizing and comparing vaccines.
- rAMTB can be an ideal PRIME!
- Recombinant Test Pathogens can facilitate vaccine development and evaluation.